

**565-Pos Board B334****S(+)-MDMA and 5-HT Evoke Dissimilar Ionic Currents in Human Serotonin Transporter-Expressing Cells**Vanessa Cabra<sup>1</sup>, Edgar Leal-Pinto<sup>1</sup>, Montserrat Samso<sup>1</sup>, Richard A. Glennon<sup>2</sup>, Louis J. De Felice<sup>1</sup>, **Jose M. Eltit<sup>1</sup>**.<sup>1</sup>Virginia Commonwealth University, School of Medicine, Department of Physiology and Biophysics, Richmond, VA, USA, <sup>2</sup>Virginia Commonwealth University, School of Pharmacy, Department of Medicinal Chemistry, Richmond, VA, USA.

Although monoamine transporters translocate substrates (neurotransmitters) across the plasma membrane, a second less studied function is their ion channel-like activity which is responsible for the passive permeation of ions through the membrane. These currents are inward at resting membrane potential and would result in cell depolarization, which may enhance presynaptic excitability, and which would help explain the elevated release of neurotransmitter associated by psychostimulants. Here we compare the ionic currents evoked by serotonin (5-HT) and S(+)-3,4-methylenedioxy-N-methylamphetamine (S(+)-MDMA, ecstasy) in mammalian cells expressing the human serotonin transporter (hSERT). 5-HT as well as S(+)-MDMA induce inward currents in hSERT-expressing cells. Although the conductances are similar, the S(+)-MDMA induced-current reversal potential is strongly shifted towards the Na<sup>+</sup> equilibrium potential, indicating a bigger contribution of Na<sup>+</sup> to the overall current compared to 5-HT current. To measure changes in Na<sup>+</sup> permeability induced by these compounds, the cells were pre-loaded with the Na<sup>+</sup> sensor Asante NaTRIUM GREEN 2 and changes in intracellular Na<sup>+</sup> concentration ([Na<sup>+</sup>]<sub>i</sub>) were monitored fluorometrically. Dose-response experiments show that 5-HT and S(+)-MDMA have similar potency (EC<sub>50</sub> ~200 nM) in elevating the [Na<sup>+</sup>]<sub>i</sub>; however, S(+)-MDMA increases [Na<sup>+</sup>]<sub>i</sub> at least 3 times with respect to 5-HT. In addition, the reconstitution of hSERT on a lipid bilayer in the presence of a Na<sup>+</sup> gradient showed channel-like activity after the application of 5-HT or S(+)-MDMA in the trans side. Taken together these results strongly suggest that S(+)-MDMA-induced current is different in composition than the 5-HT current, and moreover it must be strongly depolarizing due to its higher Na<sup>+</sup> content.

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**566-Pos Board B335****Is the Second Sodium Pump Electrogenic?****Jesús R. del Castillo**, Luz E. Thomas, Miguel A. Rocafall.

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Trans epithelial sodium transport is a process that involves Na<sup>+</sup> entry across the apical membrane of the epithelial cell following its electrochemical gradient and its active extrusion at the basolateral plasma membrane by two sodium pumps: The Na/K-pump, which depends on K<sup>+</sup>, is inhibited by ouabain and insensitive to furosemide; and the Second Sodium pump, which is K<sup>+</sup>-independent, insensitive to ouabain but inhibited by furosemide (J. Gen. Physiol. 51:303s-314s, 1968; Pflügers Archiv Eur J Physiol 316:1-25, 1970; Biochim Biophys Acta 394:281-292, 1975). Both transport mechanisms are associated with two ATPases present in the basolateral plasma membrane of the epithelial cells with similar functional characteristics, the Na<sup>+</sup>/K<sup>+</sup>-ATPase and the Na<sup>+</sup>-ATPase, respectively (Biochim Biophys Acta 1808:1684-1700, 2011; Pflügers Archiv Eur J Physiol. 316:1-25, 2012). The Na<sup>+</sup>/K<sup>+</sup>-pump is electrogenic; it exchanges 3 internal Na<sup>+</sup> by 2 external K<sup>+</sup> producing a net charge movement. The Second pump transports Na<sup>+</sup> with Cl<sup>-</sup> and water. It has been suggested that this pump could be electrogenic. The Na<sup>+</sup> charge transfer would induce Cl<sup>-</sup> movement and osmotic movement of water following the movement of both Na<sup>+</sup> and Cl<sup>-</sup> across the membrane. We have evaluated electrical parameters of the basolateral plasma membranes of MDCK cells cultured on Transwell in Ussing chambers, using amphotericin B as apical permeabilizing agent. Our results confirm that the Na<sup>+</sup>/K<sup>+</sup>-pump is electrogenic and that the Second sodium pump is electroneutral, coupling the active Na<sup>+</sup> transport to a specific Cl<sup>-</sup> movement through a particular conductive pathway.

**567-Pos Board B336****β-Exodomain Role in Na/K-ATPase α-β Subunit Interactions****Rupa R. Ram**, Albert Antolin, Natascia Vedovato, David C. Gadsby. Rockefeller University, New York, NY, USA.

We disrupted the three conserved disulfide bonds in *Xenopus* β3 (C127–C144, C154–C170, C191–C248), one at a time, using Cys to Ala/Ser mutations and coexpression with relatively ouabain-resistant (C113Y) *Xenopus* α1 in oocytes. We assessed function with two-microelectrode voltage clamp in 1 μM ouabain to silence endogenous Na/K pumps. Single C127S/A or C154S, or double C127S+C154S β3 mutants yielded steady Na/K pump currents (saturating [Ko]) and transient Na charge movements (without Ko) like those of C113Y α1 coexpressed with wild-type β3, suggesting that neither assembly, traffick-

ing, nor Na/K pump function require intact first and/or second disulfide bonds. In contrast, steady and transient pump currents were both ≥ 10 fold smaller in Na/K pumps with disrupted third disulfide bond after C113Y α1 coexpression with C191A/S β3. Similarly, in outside-out excised patches, palytoxin-induced pump-channel currents were ≥ 10 fold smaller for C113Y α1 Na/K pumps coexpressed with β3 C191S than with wild-type β3. Because in uninjected oocytes or oocytes expressing C113Y α1 alone, neither steady nor transient pump current could be detected, and palytoxin elicited no measurable current in outside-out patches, the third disulfide bond appears necessary for efficient Na/K-ATPase assembly and trafficking to the cell membrane but is not essential for assembled pump function. We disrupted salt bridges linking the αTM7-TM8 loop and β-exodomain in Na/K-ATPase structures (α1 E901-β3 K219, α1 E911-β3 R262), by coexpressing E901R(C113Y) or E911R(C113Y) α1 with wild-type β3. In both mutants, steady Na/K pump currents (at saturating [Ko]) and transient Na charge movements were like wild type; but the E901R α1 mutation selectively enhanced apparent Ko affinity ~2 fold. These results suggest that some β-exodomain structural alterations can be tolerated; but α-β subunit interface interactions closely regulate Ko binding/occlusion steps, though not the slow step that occludes/deoccludes the third Na ion. [HL36783]

**568-Pos Board B337****Protein Translocation at the Single Molecule Level****Jan Peter Birkner**, Ilja Kusters, Arnold Driessen, Antoine van Oijen.

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All proteins are synthesized in the cytoplasm. However, for many of them the cytoplasm is not their final destination. Instead, they need to be translocated across or into the plasma membrane. In bacteria, this process is mediated by the membrane-embedded protein-conducting SecYEG channel. This channel can either associate with the ribosome (co-translational translocation) or with a cytosolic motor protein, the SecA ATPase (post-translational translocation), with each the ability to provide the driving force for the translocation process across the membrane.

Even though the SecYEG system has been intensively studied, many aspects of protein translocation remain elusive. Here, we study translocation by single-molecule fluorescence imaging. We reconstitute the bacterial SecYEG into phospholipid bilayer nanodiscs and immobilize these on a functionalized glass-surface. By monitoring the interactions of the components of the Sec translocon at resolution of individual molecules we aim to provide a means for better understanding the journey of proteins into or across the membrane.

**569-Pos Board B338****Studies of the Conformational Dynamics of Ligand and Nucleotide Bound P-Glycoprotein****Jerome Ma**, Philip C. Biggin.

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A major factor contributing to the therapeutic effectiveness of a drug is whether it will be a substrate for efflux transporter proteins such as P-glycoprotein (P-gp). P-gp is an ATP-binding cassette transporter that is able to expel a remarkable range of therapeutic drugs from their target cells. Despite this, surprisingly little is known about the mechanism with which it exports drugs. Following drug binding within P-gp's transmembrane domain (TMD) binding cavity, export to the cell exterior is believed to be driven by ATP binding and/or hydrolysis at the cytoplasmic nucleotide binding domains (NBDs). We have been using atomistic molecular dynamics simulation to study the interactions of experimentally characterized ligands with the binding cavity of P-gp and their influence on P-gp NBD dynamics.

Available structures of P-gp display large separations of their NBDs. We observed tighter association of the NBDs in our simulations even in the absence of nucleotide. The degree of association and ATP binding site conformations were dependent on whether substrate or inhibitor was bound in the TMD binding cavity. In addition, conformational changes in the binding cavity coupled with ligand dynamics allowed formation of protein-ligand contacts that agreed with previous mutational studies but could not be predicted from just the crystal structure. Finally we explored the effects of ATP binding and hydrolysis to better understand how this process is coupled to substrate transport.

**570-Pos Board B339****The IC50 for Inhibition of Digoxin Transport Across Confluent Cell Monolayers of P-gp Expressing Cell Lines is Often a Function of Inhibitor Binding to Both P-gp and an Unidentified Basolateral Digoxin Uptake Transporter****Joe Bentz**.

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Previously, we reported that the P-gp substrate digoxin requires basolateral and apical uptake transporter(s) to achieve the observed efflux kinetics across MDCKII-MDR1 (Netherlands Cancer Institute) confluent cell monolayers.